18. The method according to claim 17, wherein the bacterial expression system is an *E. coli* expression system.

19. The method according to claim 13, wherein the chromatography is an anion exchange.

20. The method according to claim 19, wherein the chromatography is hydrophobic interaction.

II. IN THE CLAIMS (MARKED SHEET)

Please amend the claims as follows:

2. (Amended)

3. (Amended) The method according to claim 2, wherein sulfitolyzation comprises reacting [reduced] oxidized recombinant Troponin I with sodium [tetrathionate] sulfite.

9. (Amended) The method according to claim 6, which further comprises deprotecting the sulfhydryl groups from the purified Troponin I.[.]

13. A method of purifying Troponin I, which method comprises subjecting Troponin I comprising sulfhydryl protecting groups to chromatography to purify the sulfhydryl protected Troponin I.

15. (Amended) The method according to claim 14, wherein sulfitolyzation

comprises reacting [reduced] oxidized, denatured recombinant Troponin I with sodium [tetrathionate] sulfite.

19. The method according to claim 13, wherein the chromotography [a chromatographic support] is an anion exchange [column].

20. The method according to claim 19, wherein the chromatography is [on a] hydrophobic interaction [chromatographic support].

IV. IN THE SPECIFICATION (MARKED VERSION)

On page 2, line 24, please replace the word "tetrathionate" with - -sulfite - -.

On page 2, line 24, please replace the word "non-reducing" with - -reducing - -.

On page 3, line 16, please replace "rToponin-I" with - - recombinant Troponin - -.

On page 3, line 26, please replace "AEX" with - - - -.

On page 4, line 13, please replace "MW Stds" with - - Molecular Weight Standards - -.

On page 13, line 10, please replace "UF/DF" with - - Ultrafiltration/Diafiltration - -.

On page 4, line 10, please replace "LysC" with - - Lysate C - -.

On page 4, line 25, please replace "LC/MS" with - - Liquid Chromotography/Mass Spectoscopy - -.

On page 5, line 18, please replace "(1-6M)" with - - (1-6 M) - -.

V. REMARKS

A. Affirmation of Election

Applicants hereby affirm the election of Group I, Claims 1-9 and 13-20, for prosecution, with traverse, pursuant to 35 USC §121.

B. Specification Objections

Typographical errors and minor amendments have been made to comply with the Examiner's objections to the specification. Such corrections do not create estoppel and were not made for any reason related to patentability under 35 USC §§ 101, 102, 103, and/or 112.

C. 35 USC §112, 1ST ¶

Claims 1-9 and 13-20 stand rejected under 35 USC §112, 1st ¶, because the Examiner contends that the specification, while being enabling for sulfitolyzing and separating naturally occurring Troponin I (TnI) polypeptide, does not reasonably provide enablement for recombinant (mutant) TnI polypeptide. Applicant respectfully requests reconsideration.

To begin, it appears that the Examiner is incorrect with a basic assumption. The Examiner, as is evident from the rejection, contends that recombinant TnI is the same thing as mutant TnI. This is an incorrect assumption.

Applicants has specifically defined "recombinant TnI" on page 6, lines 5-10 of the specification. The specification provides rTnI refers to TnI prepared by a biological process. The specification further provides, in the same paragraph, that TnI is a polypeptide of about 21kD containing three cysteine residues, although the invention encompasses modified forms of TnI lacking one or two cysteine residues. A mutant TnI is a TnI that carries a specific mutation, such as, but not by way of limitation, at least one cysteine residue, but not three. Accordingly, recombinant TnI is not the same, in all embodiments, as mutant TnI. Therefore, the premise for the Examiner's rejection is incorrect.

Further, the Examiner states that the specification has not taught how to produce a recombinant TnI. Applicant respectfully requests reconsideration. On page 5, lines 14-20, Applicant specificallt sates that, in an embodiment, recombinant TnI can be expressed in a bacterial system. Further, Applicants proceeds to explain how, in an embodiment, the recovery of TnI from inclusion bodies is performed. On page 6, lines 19-28,

Applicants further disclose numerous reference publications detailing how to produce recombinant DNA (which would include recombinant TnI). Furthermore, on page 14, last paragraph, Applicant incorporated these publications by reference. Therefore, there is ample teaching of how to construct and express recombinant TnI. Likewise, the specification specifically incorporates by reference recombinant DNA techniques that would include a mutant TnI, as is defined in the specification.

The Examiner's contention that the specification is not enabling for chemically protecting sulfhydryl side chains is misplaced. The specification, at page 7, line 26 to page 8, line 12, specifically defines a "sulfhydryl protecting group or "cysteine protecting group" as a reversibly bound chemical group which prevents formation of the intra- and intermolecular disulfide bonds, but dos not interfere with the process of protein purification. The specification goes on to state that a preferred embodiment is/are sulfate group(s) bound through sulfitolyzation with sodium tetrathionate. In fact, the specification is abundantly clear that the invention contemplates numerous other embodiments as protecting groups, such as, without limitation, disulfide compounds, alkylalkanethiosulfonates, and/or the like. Further, the specification teaches other sulfhydryl-reactive chemistries that may be used with potential utility in simplifying toponin I purification, recovery and storage in embodiments of the present invention. These other chemistries include, but nit limited to, cyanylation, aminoethyation, reaction with compounds containing maleimide functional groups, and the like. Accordingly, Applicant has disclosed and enabled numerous chemistries that are common in the art and can be applied to the wide range of naturally occurring, recombinant, and/or mutant TnI. Moreover, Applicant specifically states that it is preferable to denature naturally

occurring, recombinant, and/or mutant TnI prior to protecting sulfhydryl groups and/or applying chromatographic methods. See page 8, lines 13-15. Therefore, as long as the naturally occurring, recombinant and/or mutant TnI has at least a Cysteine, protecting the sulhydryl groups of the present invention can occur.

Likewise, Applicants has provided enablement for the refolding of TnI into a bioactive confirmation. To begin, Applicant has defined "refolding" on page 5, lines 21 and 22 to mean changes in the three-dimensional conformation of the protein, which restore the protein's biological activity, including its antiangiogenic properties. Applicant proceeds to give a non-limiting example of protein refolding of TnI on page 5, line 23 to page 6, line 4. Accordingly, an contrary to the Examiner's assertions, Applicant has provided an enabling disclosure for refolding TnI protein, a skill that is common in the art.

The Examiner then proceeds to a Wands analysis. However, such an analysis is not needed. Applicants have defined the invention. The Examiners primary rejection appears to be that not all mutants of TnI have been enabled. Applicants respectfully request that the Examiner consider the definitions for the terms used in the specification. In an embodiment, the present invention is a method of preparing Troponin I, which method comprises protecting free sulfhydryl groups of Troponin I under reducing conditions. Another embodiment of a method of the present invention relates to purifying Troponin I after reversibly protecting the free sulfhydryl groups, purification by chromatography, and deprotection of the sulfhydryl groups to yield a highly purified protein. Applicants respectfully request reconsideration of the rejection in light of this response. The Examiner's rejection does not recognize that the first embodiment listed in

the preceding paragraph only requires a Cysteine residue on the strand. The Examiner is adding limitations to Applicant's broadest claim. Accordingly, the Examiner's rejection of Claim 1 is wrong. Likewise, Applicant has provided ample evidence, taken from the specification, to illustrate that the various embodiments of the invention are enabled. Therefore, Applicant respectfully requests reconsideration of the rejection in light of this response.

D. 35 USC §112, 2nd ¶

Claims 1, 3, 4, 8, 9, and 13-20 stand rejected under 35 USC §112, 2nd ¶, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

The Examiner contends that Claim 1 is unclear as to what and with what the sulhydryl groups are protecting. Applicant has addressed this issue in response to Claim 1. The specification, at page 7, line 26 to page 8, line 12, specifically defines a "sulfhydryl protecting group or "cysteine protecting group" as a reversibly bound chemical group which prevents formation of the intra- and intermolecular disulfide bonds, but dos not interfere with the process of protein purification. The specification goes on to state that a preferred embodiment is/are sulfate group(s) bound through sulfitolyzation with sodium tetrathionate. In fact, the specification is abundantly clear that the invention contemplates numerous other embodiments as protecting groups, such as, without limitation, disulfide compounds, alkylalkanethiosulfonates, and/or the like. Further, the specification teaches other sulfhydryl-reactive chemistries that may be used with potential utility in simplifying toponin I purification, recovery and storage in

embodiments of the present invention. These other chemistries include, but nit limited to, cyanylation, aminoethyation, reaction with compounds containing maleimide functional groups, and the like. Accordingly, Applicant has disclosed and enabled numerous chemistries that are common in the art and can be applied to the wide range of naturally occurring, recombinant, and/or mutant TnI. Moreover, Applicant specifically states that it is preferable to denature naturally occurring, recombinant, and/or mutant TnI prior to protecting sulfhydryl groups and/or applying chromatographic methods. See page 8, lines 13-15. Therefore, as long as the naturally occurring, recombinant and/or mutant TnI has at least a Cysteine, protecting the sulhydryl groups of the present invention can occur. Claim 1 only recites that the sulfhydryl groups are protected. The specification then provides enablement for that language. A correct analysis by the Examiner would be to determine if the prior art disclosed protecting sulfhydryl groups of Troponin I. If not, there can be no rejection. The law is clear, the second paragraph of § 112 contains two requirements: "first, [the claim] must set forth what 'the applicant regards as his invention,' and second, it must do so with sufficient particularity and distinctness, i.e., the claim must be sufficiently 'definite.'" ***Solomon v. Kimberly-Clark Corp.***, 216 F.3d 1372, 1377, 55 USPQ2d 1279, 1282 (Fed. Cir. 2000). In determining whether the claim is sufficiently definite, we must analyze whether "one skilled in the art would understand the bounds of the claim when read in light of the specification." ***Personalized Media Communications, LLC v. Int'l Trade Comm'n***, 161 F.3d 696, 705, 48 USPQ2d 1880, 1888 (Fed. Cir. 1998). Here, Applicant has stated an invention, supported by the specification, that one of ordinary skill in the art would comprehend. Therefore, Applicant respectfully requests reconsideration.

The Examiner contends that Claim 3 is unclear for antecedent basis. Applicant has amended the claims. Applicant respectfully requests reconsideration.

The Examiner contends that Claim 8 is indefinite as to whether the Troponin I remains protected in the non-reducing conditions of Claim 8 since Claim 1 and Claim 6 indicate protection under reducing conditions. Applicant requests that the Examiner read the claims as limited by Claim 8. It has long been the law that dependent claims must further limit claims from which they depend. Applicant specifically made Claim 8 dependent upon Claim 6, which is dependent upon Claim 1. Therefore, Claim 8 has all the limitations of Claim 1 and Claim 6. Claim 8 further limits Claim 6 by purifying the Troponin I under non-reducing conditions. Applicant respectfully requests reconsideration.

Claim 9 has been amended.

Claim 13 has been amended.

Claim 15 has been amended.

Claim 19 has been amended.

Claim 20 has been amended.

E. 35 USC §103(A) REJECTION

Claims 1-9 and 13-20 stand rejected under 35 USC §103(a) as being obvious over an artciel to Fujita-Baket et al. (J. Biochem., (1993) 114, pp. 438-444), taken with an article to Reiffert et al. (Eur. J. Biochem., (1999) 261, pp. 40-47), an article to Gushoff et al (J. Bacteriol., (1975) 122, pp. 599-605), and US pat. No. 4,734,362 to Hung et al.

However, the Examiner has failed to establish a prima facie case of obviousness. Applicants respectfully requests reconsideration.

It has long been the law that an obviousness determination requires an analysis of four factors. The factors are 1) scope and content of prior art; 2) the differences between the prior art and the claims at issue; 3) the level of ordinary skill in the art; and, 4) objective evidence of non-obviousness. ***See Graham v. John Deere Co.***, 383 U.S. 1, 17, 148 USPQ 459, 467 (1966). Here, the Examiner did not consider these factors.

To ascertain the scope of the prior art, a court examines "the field of the inventor's endeavor," ***Shatterproof Glass Corp. v. Libbey-Owens Ford Co.*** , 758 F.2d 613, 620, 225 USPQ 634, 638 (Fed. Cir. 1985), and "the particular problem with which the inventor was involved," ***Stratoflex, Inc. v. Aeroquip Corp.***, 713 F.2d 1530, 1535, 218 USPQ 871, 876 (Fed. Cir. 1983) (***quoting In re Wood***, 599 F.2d 1032, 1036, 202 USPQ 171, 174 (CCPA 1979)), at the "time the invention was made," see 35 U.S.C. Section 103(a). Here, the Examiner contends that the Fujita article teaches purification of Troponin I from *E.* coli expression systems using a Q-Sepharose column and phenyl-sepharose column. The Examiner further contends that the Reiffert article teaches purification of recombinant Troponin I from bacterial culture using ion exchange and a reverse phase hydrophobic interaction column. The Examiner further contends that the Grushoff article teaches chromatographic purification of bacterial protein utilizing tetrathionate to protect the protein by blocking the sulfhydryl groups. Lastly, the Examiner contends that the '362 patent teaches a deprotecting method.

The Examiner then, without any teaching or motivation, combines these four references and states that Applicant's invention is obvious. However, the Examiner did not consider his own statements.

Without even considering any further teachings of the prior art, the Examiner has admitted vast differences between the prior art and Applicant's invention.

First, on page 11, paragraph 3, the Examiner admits that the article to Fujita does not teach purifying recombinant TnI polypeptides. In fact, the Fujita article is a prior art method of purification rabbit skeletal muscle troponin complex. The article specifically states, at page 439, that a solution it used to overcome the disulfide bonds was to make a mutant TnI without 2 of the three cysteine residues. Accordingly, the Fujita article is teaching away from Applicants invention. The Fujita article teaches removing the Cysteine residues to prevent disulfide bonding, rather than as applicant protects the Cysteines.

Likewise, the Examiner admits on pages 11 and 12 that the Grushoff does not teach the purification of Troponin I. In fact, the Grushoff article teaches a method for purification of streptococcal NADase. There is no teaching for Troponin I.

Further, the Reiffert article does not teach protecting the sulfhydryl groups. The Reiffert article, like The Fujita article teaches substitution or removal of Cysteine residues. Therefore, the Reiffert article teaches away from Applicant's invention.

Lastly, the '362 patent discloses a completely different process than Applicant's invention. The '362 patent claims a process for recovering refractile body recombinant protein from microorganisms comprising (1) disrupting the host cells containing said refractile body recombinant protein; (b) solubilizing said refractile body; (c) acid

acylating free amino groups contained in said recombinant protein; (d) separating said recombinant protein in N-acid acylated form; and (e) recovering said recombinant protein. However, most importantly, the '362 patent requires reducing the protein. See the '362 patent, Col. 8, ll. 51-61. Accordingly, Applicant's invention is not disclosed.

To establish a prima facie case of obviousness, the Board must, inter alia, show "some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings of the references." In re Fine, 837 F.2d 1071, 1074, 5 USPQ2d 1596, 1598 (Fed. Cir. 1988). "The motivation, suggestion or teaching may come explicitly from statements in the prior art, the knowledge of one of ordinary skill in the art, or, in some cases the nature of the problem to be solved." Kotzab, 217 F.3d at 1370, 55 USPQ2d at 1317.

The Examiner has only gone to the prior art and identified each element. It is a well accepted principle that the identification in the prior art of each individual part claimed is insufficient to defeat patentability of the whole claimed invention. See id. Rather, to establish obviousness based on a combination of the elements disclosed in the prior art, there must be some motivation, suggestion or teaching of the desirability of making the specific combination that was made by the applicant. ***See In re Dance***, 160 F.3d 1339, 1343, 48 USPQ2d 1635, 1637 (Fed. Cir. 1998); ***In re Gordon***, 733 F.2d 900, 902, 221 USPQ 1125, 1127 (Fed. Cir. 1984). Determination of obviousness can not be based on the hindsight combination of components selectively culled from the prior art to fit the parameters of the patented invention. There must be a teaching or suggestion within the prior art to look to particular sources of information, to select particular elements, and to combine them in the way they were combined by the inventor. ***See***

Heidelberger Druckmaschinen AG v. Hantscho Commercial Prods., Inc. , 21 F.3d 1068, 1072, 30 USPQ2d 1377, 1379 (Fed. Cir. 1994) ("When the patented invention is made by combining known components to achieve a new system, the prior art must provide a suggestion or motivation to make such a combination."); ***Northern Telecom, Inc. v. Datapoint Corp.*** , 908 F.2d 931, 935, 15 USPQ2d 1321, 1324 (Fed. Cir. 1990) (the prior art must suggest to one of ordinary skill in the art the desirability of the claimed composition); ***Interconnect Planning Corp. v. Feil*** , 774 F.2d 1132, 1143, 227 USPQ 543, 551 (Fed. Cir. 1985). Here, the Examiner has only vaguely stated, on page 12, last paragraph, that "Given the above motivation... [it would have been obvious]. However, nothing above that paragraph provided any motivation or any teaching to combine the references. Therefore, the rejection is improper.

Our case law makes clear that the best defense against the subtle but powerful attraction of a hindsight-based obviousness analysis is rigorous application of the requirement for a showing of the teaching or motivation to combine prior art references. ***See , e.g. , C.R. Bard, Inc. v. M3 Sys., Inc.*** , 157 F.3d 1340, 1352, 48 USPQ2d 1225, 1232 (Fed. Cir. 1998) (describing "teaching or suggestion or motivation [to combine]" as an "essential evidentiary component of an obviousness holding"); ***In re Rouffet*** , 149 F.3d 1350, 1359, 47 USPQ2d 1453, 1459 (Fed. Cir. 1998) ("the Board must identify specifically . . . the reasons one of ordinary skill in the art would have been motivated to select the references and combine them"); ***In re Fritch*** , 972 F.2d 1260, 1265, 23 USPQ2d 1780, 1783 (Fed. Cir. 1992) (examiner can satisfy burden of obviousness in light of combination "only by showing some objective teaching [leading to the combination]"); ***In re Fine*** , 837 F.2d 1071, 1075, 5 USPQ2d 1596, 1600 (Fed.

Cir. 1988) (evidence of teaching or suggestion "essential" to avoid hindsight); ***Ashland Oil, Inc. v. Delta Resins & Refractories, Inc.*** , 776 F.2d 281, 297, 227 USPQ 657, 667 (Fed. Cir. 1985) (district court's conclusion of obviousness was error when it "did not elucidate any factual teachings, suggestions or incentives from this prior art that showed the propriety of combination"). ***See also Graham*** , 383 U.S. at 18, 148 USPQ at 467 ("strict observance" of factual predicates to obviousness conclusion required). Combining prior art references without evidence of such a suggestion, teaching, or motivation simply takes the inventor's disclosure as a blueprint for piecing together the prior art to defeat patentability--the essence of hindsight. ***See, e.g., Interconnect Planning Corp. v. Feil***, 774 F.2d 1132, 1138, 227 USPQ 543, 547 (Fed. Cir. 1985) ("The invention must be viewed not with the blueprint drawn by the inventor, but in the state of the art that existed at the time."). In this case, the Examiner has fallen into that hindsight based trap. Here, there was no suggestion or motivation pointed out by the Examiner. In fact, the art cited did more to teach away from Applicant's invention, such as by removing the Cysteine residues.

Accordingly, in light of the Examiners deficient 103 rejection and the lack of any suggestion or motivation to combine these abstract pieces of prior art, Applicant respectfully requests reconsideration of the rejection.

F. Double Patenting and Provisional-Type Double Patenting

Applicant has abandoned the other application. Therefore, Applicant respectfully requests reconsideration of the rejection.

VI. CONCLUSION

The application is believed in a condition for allowance and Applicants respectfully request such action. Applicant respectfully requests an interview with the Examiner. Please call the below undersigned attorney for any assistance in securing allowance of this application, at (302) 933-4034. Please charge deposit account number 02-2334 for any required fees.

Date: October 3, 2002

Sincerely,

William P. Ramey, III
Reg. No. 44,295

AKZO Nobel
Intervet, Inc.
405 State Street
Millsboro, DE 19966
(302) 933-4034 telephone
(302) 934-4035 facsimile